Effects of ectomycorrhizae and solution pH on [^{15}N]ammonium uptake by coniferous seedlings

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Received March 28, 1984[3]
Accepted August 7, 1984

RYGIEWICZ, P. T., C. S. BLEDSOE, and R. J. ZASOSKI. 1984. Effects of ectomycorrhizae and solution pH on [^{15}N]ammonium uptake by coniferous seedlings. Can. J. For. Res. **14**: 885–892.

Effects of mycorrhizae and solution pH on ammonium uptake and fluxes of hydrogen, calcium, and potassium in solution culture were determined using three Northwest coniferous species: Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). The ectomycorrhizal fungus was *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél. Ammonium uptake rates decreased with decreasing pH and were accompanied by decreasing H^+ release to the external solution. The mycorrhizal fungus *H. crustuliniforme* enhanced ammonium uptake and generally released fewer hydrogen ions per ammonium ion taken up than did nonmycorrhizal roots. This altered stoichiometry in mycorrhizal seedlings could mediate pH changes in the rhizosphere of soil-grown plants. During ammonium uptake, calcium ions were released at low pH and taken up at higher pH. Potassium efflux occurred at all hydrogen levels, with less efflux at higher pH. The mycorrhizal association generally increased calcium uptake or decreased calcium and potassium efflux rates compared with nonmycorrhizal seedlings. This research demonstrates that the ectomycorrhizal fungus *Hebeloma crustuliniforme* can significantly improve ammonium acquisition, a major growth-limiting nutrient in most Pacific Northwest forests, and that pH strongly affects ammonium uptake.

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Les auteurs ont étudié les effets des mycorhizes et du pH de la solution de culture sur l'absorption de l'ammonium et sur les mouvements de l'hydrogène, du calcium et du potassium, en utilisant trois espèces conifériennes du nord-ouest américain: le sapin de Douglas (*Pseudotsuga menziesii* (Mirb.) Franco), l'épinette de Sitka (*Picea sitchensis* (Bong.) Carr.) et la pruche de l'ouest (*Tsuga heterophylla* (Raf.) Sarg.). Le champignon ectomycorhizien était l'*Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél. Les taux d'absorption de l'ammonium diminuent avec une diminution du pH et sont accompagnés d'une diminution du relâchement d'ion H^+ dans la solution. Le champignon mycorhizien *H. crustuliniforme* augmente l'absorption de l'ammonium et on retrouve généralement dans ce cas moins d'ions hydrogène par ion ammonium absorbé qu'avec les racines nonmycorhiziennes. Cette modification de la stoechiométrie avec les plants mycorhizés pourrait occasionner des changements de pH dans la rhizosphère de plants cultivés en sol. Pendant l'absorption de l'ammonium, des ions calcium sont relâchés aux faibles pH et absorbés aux pH élevés. Une sortie de potassium a lieu à toutes les concentrations d'hydrogène, avec diminution aux pH élevés. L'association mycorhizienne augmente généralement l'absorption du calcium ou diminue les taux de sortie du calcium et du potassium comparativement aux plantules nonmycorhizées. Cette recherche démontre que le champignon mycorhizien *H. crustuliniforme* améliore significativement l'absorption de l'ammonium, un nutriment majeur susceptible de limiter la croissance dans la majorité des forêts du nord-ouest américain sur la côte du Pacifique et que le pH affecte fortement l'absorption de l'ammonium.

[Traduit par le journal]

Introduction

Nitrogen availability is the most important nutrient factor limiting forest growth in the Pacific Northwest of North America (Date 1973), where coniferous species respond positively to nitrogen fertilization (Gessel and Atkinson 1979). Acidic Pacific Northwest forest soils commonly have reduced nitrification and mineralization rates, which makes ammonium (NH_4^+) the prevalent form of inorganic nitrogen (Thompson and Troeh 1978). In the carbon-rich, nitrogen-poor forest soils, coniferous roots compete with microbes and other plant roots for NH_4^+ present at low levels. Under these circumstances, ectomycorrhizae may enhance NH_4^+ acquisition by coniferous roots. Study of ammonium acquisition is complicated by several factors.

Protons released during NH_4^+ uptake substantially affect ambient pH (Nie 1981; van den Driessche 1978). These pH changes can alter subsequent uptake of NH_4^+ and other ions, and affect availability of nutrients. Biochemical and biophysical processes internal and external to the cell which control ambient and cellular pH have been shown to vary with external pH (Rufty et al. 1982). Therefore, external pH should be maintained at constant values while studying NH_4^+ uptake.

When plants are mycorrhizal, nitrogen uptake rates will be influenced by the inherent uptake rates of both the mycobiont and host. Most ectomycorrhizal fungi are considered to be acidophyllic and grow more with NH_4^+ as a N source than when NO_3^- is supplied (Littke et al. 1984; Lundberg 1970; Slankis 1974). Coniferous species also generally prefer NH_4^+ but data are more variable (van den Driessche 1971; Krajina et al. 1973; McFee and Stone 1968). Interpretation of preference for nitrogen sources is complicated by uncontrolled pH during the uptake period. Few studies have examined pH effects on NH_4^+ uptake under conditions where external pH was maintained at a predetermined constant level.

This study was designed to test the influence of ectomycorrhizae and solution pH on NH_4^+ uptake by coniferous seedlings in solutions where pH was maintained at a constant value. Nitrate uptake was examined in another study (Rygiewicz et al. 1984). Because soils of the Pacific Northwest

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[3]Revised manuscript received August 2, 1984.

and other forest regions have widely varying pH's, an understanding of pH–NH_4^+ interactions under controlled conditions could provide valuable insights into the nitrogen nutrition of both mycorrhizal and nonmycorrhizal coniferous species. Concerns of acid rain and other acidification processes would also benefit from this information.

Materials and methods

Plant material

Seedlings (low elevation seed sources) of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) were grown in peat–vermiculite–perlite (1:1:1 volume basis) planting medium in plastic tubes (2.5 × 16.5 cm) in a greenhouse. Seedlings were fertilized weekly with a complete fertilizer solution using ammonium nitrate as the nitrogen source (Rygiewicz 1983). Four to six months after germination, half of the seedlings were inoculated with the ectomycorrhizal fungus *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél (U.S. Forest Service, Forestry Sciences Laboratory, Corvallis, OR, stock No. S-166) using the technique of Littke et al. (1980). When the seedlings were 9 months old and mycorrhizae had formed, roots were gently washed free of the planting medium. Seedlings were actively growing and bud set had not yet occurred. Root tips of mycorrhizal seedlings were approximately 40–50% mycorrhizal infected, compared with less than 1% infection for uninoculated controls. The contaminant for control seedlings was probably *Telephora terrestris*.

Experimental design

The three coniferous species mentioned above, with and without mycorrhizae, were tested at six pH values: 3.1, 3.9, 4.7, 5.5, 6.3, and 7.1. Each treatment was replicated four times on pairs of seedlings, and all factors were fully randomized. A total of 288 seedlings was used.

Pretreatment

Washed roots of intact seedlings were pretreated for 48 h in dilute nutrient solution which contained micronutrients (1:100 dilution of Johnson's solution, Epstein (1972)) and macronutrients (0.04 m*M* NH_4Cl, 0.01 m*M* K_2HPO_4, 0.4 m*M* $CaCl_2$, and 0.01 m*M* $MgSO_4 \cdot 7H_2O$). During the pretreatment, solution pH was initially the same as the subsequent uptake solution pH, but was not maintained at a constant level. Solutions were aerated and maintained at 18°C in a temperature-controlled water bath. Seedlings received supplemental lighting with a high pressure sodium lamp, 16 h light and 8 h dark.

Apoplastic adsorption

Prior to the uptake period, seedling roots were placed in preuptake solution (see next section) for 15 min to allow CW + FS (cell wall + free space) exchange to occur. The half time for monovalent cation exchange in the CW + FS in coniferous roots was estimated to be 2 min (Rygiewicz and Bledsoe 1984). Thus equilibration with a preuptake solution of equal ionic strength to the uptake solution minimized changes in pH and ionic concentration owing to apoplastic reactions during the initial minutes of the subsequent uptake period.

Uptake and apoplastic desorption

Roots of intact seedlings were transferred to uptake solutions (Table 1) for 3 h. Solutions were aerated continuously with CO_2-free air and maintained at 18°C with supplemental lighting. The pH was maintained by the addition of 0.01 *N* NaOH with an automatic pH titrating machine (pH stat). A concentration of 1.4 m*M* ammonium (99% enrichment of stable isotope ^{15}N) was chosen to maximize NH_4^+ uptake. For Douglas-fir seedlings grown in solution culture, optimum NH_4^+ concentration was about 1.5 m*M* NH_4^+ (Littke 1982). After each uptake period, roots were placed in nitrogen-free uptake solutions at 2°C for 15 min to desorb NH_4^+ from the CW + FS. Ammonium uptake rates should thus only measure nitrogen taken up across cellular membranes.

TABLE 1. Composition of uptake and preuptake solutions with added micronutrients (as indicated in the Materials and methods, Pretreatment section)

Compound	Concentration (m*M*)	Ion	Ion concentration ($\mu Eq \cdot L^{-1}$)
$^{15}[NH_4]_2SO_4$	0.7	NH_4^+	1400
KCl	0.4	K^+	400
$CaCl_2$	0.4	Ca^{2+}	800
Na_2HPO_4	0.03	$H_2PO_4^-$*	30
$MgSO_4 \cdot 7H_2O$	0.01	Mg^{2+}	20

*This is the predominant ionic species in the pH range 3.1–6.3, while at pH 7.1 both $H_2PO_4^-$ and HPO_4^{2-} are present.

Analysis

Seedlings were separated into roots and shoots, oven dried, and weighed. Roots and tops of Douglas-fir and roots of spruce and hemlock seedlings were ground. Nitrogen was determined by the microKjeldahl method of Parkinson and Allen (1975) modified to include NO_3^- (Ekpete and Cornfield 1964), followed by steam distillation (Bremner 1965). The isotope ^{15}N was measured by mass spectrometry at Los Alamos National Laboratory.

Uptake solutions were sampled at the beginning and end of the uptake period. Uptake or release of Ca^{2+} and K^+ was calculated as the difference between initial and final nutrient concentrations of the uptake solution determined by atomic absorption. Release of H^+ from seedling roots was assumed to equal the amount of NaOH dispensed by the autoburette during the uptake period. In Figs. 1, 2, 4, 5, and 6, efflux of ions is presented as a negative value, while influx of ions is presented as a positive value.

Statistical analyses

Analyses of covariance were conducted to determine the significant effects of three major variables on nutrient fluxes: (*i*) presence of mycorrhizae; (*ii*) solution pH; and (*iii*) coniferous species, with root and total seedling dry weights and R:S ratio (root:shoot) included as covariates (SPSS, Statistical Package for the Social Science, Nie et al. (1975)). Duncan's new multiple range test (Steel and Torrie 1960) was used to identify significant differences among individual treatment means.

Presentations of the results were facilitated by separating the coniferous species and analyzing each species for mycorrhizal effects. In the absence of significant differences, data for nonmycorrhizal and mycorrhizal seedlings were combined within a species. If species effects were not significant, seedling types (mycorrhizal and nonmycorrhizal) and species were combined. When species effects were present for one seedling type but not for the other, data were presented for each species noting the differential mycorrhizal effect. In the general sense, any results that have $p \leq 0.05$ will be regarded as significant, the more specific terms highly significant, and very highly significant will be used for results where $p \leq 0.01$ and $p \leq 0.001$, respectively.

Results

Nutrient fluxes showed significant three-way interactions between mycorrhizae, coniferous species, and solution pH. Thus, the fungus affected nutrient fluxes for each coniferous species differently and to varying extents depending upon solution pH. The interactions also indicated that pH affected nutrient uptake differently for each coniferous species. These significant interactions required that the experimental design be divided into component parts for analysis to accurately assess treatment effects. Root dry weight was selected as the most useful seedling descriptor upon which to express flux data (Rygiewicz 1983).

There was little variation in seedling biomass within a spe-

TABLE 2. Seedling characteristics for mycorrhizal (+) and uninoculated control (−) plants. Values are means of 24 replicates ± SE, except potassium and calcium values which are means of 6 replicates ± SE

Coniferous species	Mycorrhizal status	Seedling dry weight (g)	Root:shoot	Nutrient concentration in roots		
				%N	%K	%Ca
Douglas-fir	+	0.82±0.07	0.89±0.02	0.74±0.01	0.30±0.02	0.12±0.01
	−	0.83±0.06	0.76±0.01	0.63±0.01	0.24±0.01	0.13±0.03
Hemlock	+	0.33±0.01	1.0±0.01	0.84±0.01	0.30±0.01	0.13±0.02
	−	0.34±0.01	0.79±0.02	0.72±0.01	0.31±0.02	0.19±0.07
Spruce	+	2.2±0.09	0.78±0.01	0.75±0.01	0.68±0.03	0.28±0.08
	−	1.4±0.07	0.66±0.01	0.72±0.01	0.61±0.02	0.28±0.06

TABLE 3. Net cation uptake or efflux for three coniferous species. Values (microequivalents per grams of dry weight root per hour) are means for all pH levels combined. Nonmycorrhizal (NON) and mycorrhizal (MYC) data for Ca^{2+} and K^+ were combined since no significant difference was found between nonmycorrhizal and mycorrhizal seedling types. The mycorrhizal effect was significant for NH_4^+ and H^+ and flux data were not pooled

Coniferous species	Ammonium			Hydrogen ion			Calcium ($\bar{X}$)	Potassium ($\bar{X}$)	Σ, measured flux[a]		
	NON	MYC	$\bar{X}$	NON	MYC	$\bar{X}$			NON	MYC	$\bar{X}$
Hemlock	2.5*	5.0*	3.8	−7.3	−10.0	−8.6	−13.2	−48.0	−80.0	−52.2	−66.0
Spruce	5.5*	7.0*	6.4	−8.3*	−5.8*	−7.1	−5.8	−12.7	−22.7	−15.9	−19.2
Douglas-fir	4.2*	8.0*	6.1	−6.2	−9.6	−7.9	−14.2	−25.9	−47.2*	−36.6*	−41.9
$\bar{X}$	4.2	6.6	5.4	−7.2	−8.4	−7.9	−11.1	−28.9	−49.7	−34.9	−42.5

NOTE: The asterisk (*) indicates ion flux between nonmycorrhizal and mycorrhizal seedling types within the coniferous species is significantly different ($p \leq 0.05$).
[a] $\Sigma = NH_4^+$ uptake + H^+ flux + Ca^{2+} flux + K^+ flux, where efflux is a negative number and uptake is a positive number.

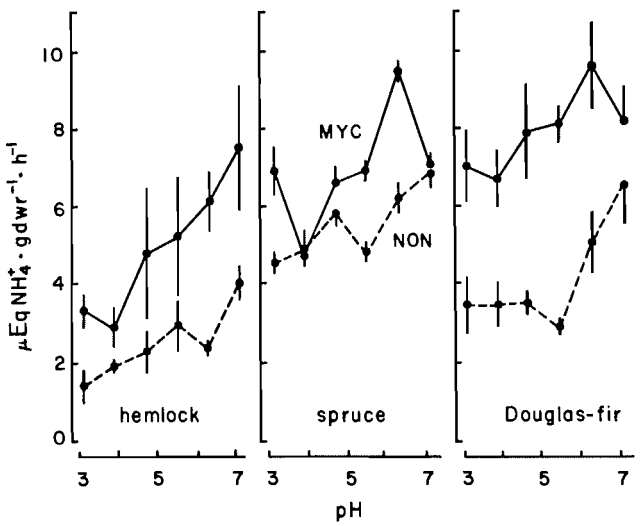

FIG. 1. Ammonium uptake by nonmycorrhizal (NON) and mycorrhizal (MYC) coniferous seedlings as a function of solution pH. Data points are means of four replications with standard errors indicated.

cies as indicated by analyses of covariance. Plants with smaller root systems, larger total dry weights, or different R:S ratios did not take up significantly different amounts of nutrients simply because they were slightly smaller or larger. No seedling parameter was consistently related to individual nutrient fluxes within mycorrhizal or nonmycorrhizal treatments. Between mycorrhizal and uninoculated control seedlings, seedling total dry weights were similar (Table 2). Mycorrhizal infection increased the R:S ratio about 20% and increased root nitrogen about 12%. Root potassium and calcium contents were not affected. As expected, seedling characteristics for Douglas-fir, hemlock, and spruce were different (Table 2), and these differences were considered in the statistical treatment of the data.

Ammonium uptake

Both increasing pH and presence of mycorrhizae significantly increased NH_4^+ uptake rates for all species (Fig. 1). Across seedling type and species, mean uptake rates increased from 1 to 9 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ (gdwr, grams of dry weight root) as pH rose from 3.9 to 7.1. At all pH levels, mycorrhizal uptake rates exceeded or equaled rates for nonmycorrhizal seedlings. In addition, a significant interaction was found between pH and seedling type.

Mean nutrient flux data for all species and both mycorrhizal conditions are shown in Table 3 for all pH levels combined. Among nonmycorrhizal seedlings, species was a very highly significant factor affecting NH_4^+ uptake rates. Hemlock seedlings had the lowest mean NH_4^+ uptake rate; the Douglas-fir rate was intermediate, and the spruce rate was highest. Among mycorrhizal seedlings, the species was also a significant factor. The mean hemlock NH_4^+ uptake rate was again lowest, but Douglas-fir and spruce rates were similar. The highest rate for hemlock at any pH was only equal to the lowest Douglas-fir rate (Fig. 1). While the effect of the mycorrhizal fungus was to increase ammonium uptake rates, the mycorrhizal effect did not change the relative rank of each species.

Over the pH range, no pH optimum was observed for the nonmycorrhizal seedlings or for mycorrhizal hemlock. In contrast, both mycorrhizal spruce and Douglas-fir seedlings absorbed NH_4^+ at increased rates as pH increased to 6.3; however, at pH 7.1 uptake rates were decreased. Because this decrease was not statistically significant for Douglas-fir, uptake experiments were repeated at pH 6.3 and 7.1. Although both experiments showed a decrease in uptake rate at pH 7.1, this decrease was not significant. Thus mycorrhizal spruce seedlings may have a lower pH optimum than nonmycorrhizal

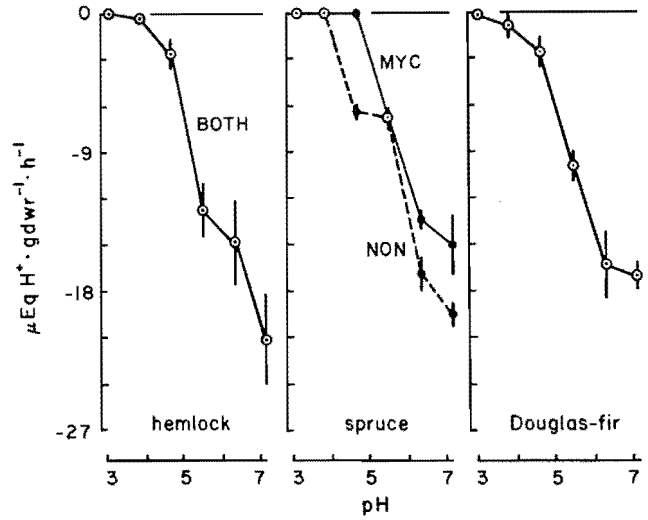

FIG. 2. Flux of H^+ during NH_4^+ uptake as a function of pH. Nonmycorrhizal and mycorrhizal data for hemlock and Douglas-fir were combined (BOTH); spruce data were not combined (NON, MYC). All other conventions as in Fig. 1.

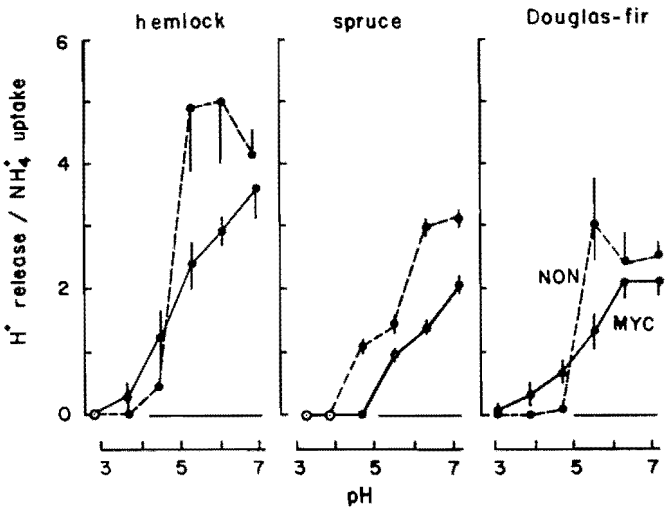

FIG. 3. The ratio of H^+ flux to NH_4^+ flux as a function of pH. All conventions as in Figs. 1 and 2.

ones, while Douglas-fir data are only suggestive of a lowered pH optimum.

To determine whether NH_4^+ uptake rates were underestimated by the rapid translocation of ^{15}N to seedling tops during the 3-h uptake, ^{15}N translocation rates of both mycorrhizal and nonmycorrhizal Douglas-fir seedlings were compared. Translocation had little effect on uptake rates, since a maximum of only 4.2% of the nitrogen taken up was found in the shoots of both seedling types. There was no pH effect on nitrogen translocation rates, and average translocation rates were not significantly different between nonmycorrhizal and mycorrhizal seedlings, respectively, across the pH range (0.10 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1} \pm 0.05$ and 0.35 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1} \pm 0.14$).

Ion fluxes during NH_4^+ uptake

Hydrogen ion

During NH_4^+ uptake, H^+ ions were released into the external solution (Fig. 2). When H^+ release rates were compared among nonmycorrhizal or mycorrhizal seedlings, there was no significant coniferous species effect. For hemlock and Douglas-fir, since there was no mycorrhizal effect, rates for the two seedling types were combined (Fig. 2). Because differences in H^+ release rates between nonmycorrhizal and mycorrhizal spruce seedlings were very highly significant, data were not combined. Mycorrhizal spruce generally released H^+ at slower rates than nonmycorrhizal spruce.

For all species, a very highly significant increase in H^+ release was observed as solution pH increased. Little or no H^+ efflux occurred at pH 3.1. At pH 3.9, there was H^+ efflux from both Douglas-fir and hemlock seedling roots; at pH 5.5 and above, seedlings in all treatments released H^+.

Because H^+ efflux may be related to fluxes of other ions, particularly NH_4^+, we calculated rates of H^+ efflux relative to NH_4^+ (H^+:NH_4^+ ratios, Fig. 3). These ratios demonstrate that spruce and Douglas-fir mycorrhizal seedlings released significantly fewer H^+ per NH_4^+ taken up than did nonmycorrhizal seedlings. Hemlock data were more variable, but at higher pH mycorrhizal hemlock also released fewer H^+ per NH_4^+ taken up. The hemlock and Douglas-fir patterns were strikingly similar (Fig. 3). The H^+:NH_4^+ ratio increased with increasing solution pH for all three coniferous species and both seedling types. This trend was particularly evident above pH 4.7 for hemlock which released more H^+ per NH_4^+ taken up than either Douglas-fir or spruce.

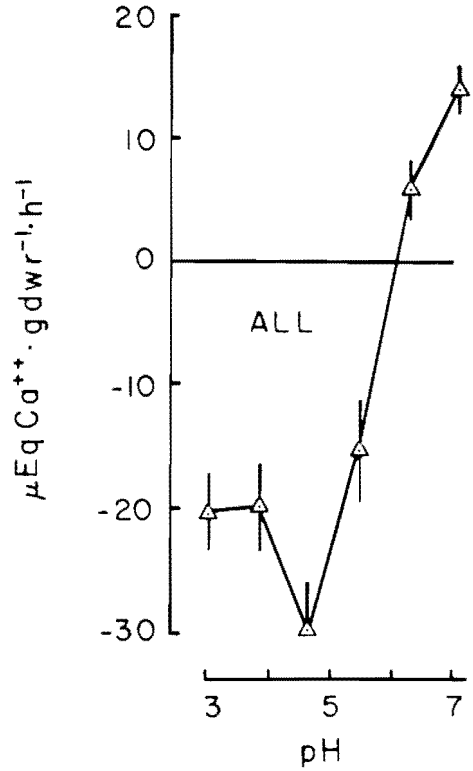

FIG. 4. Flux of Ca^{2+} during NH_4^+ uptake as a function of pH, all species combined (ALL, no significant differences among coniferous species or between nonmycorrhizal and mycorrhizal seedlings).

Calcium ion

Neither coniferous species nor mycorrhizae significantly affected Ca^{2+} fluxes, but solution pH had a very highly significant effect. Mean Ca^{2+} fluxes are pooled data for all coniferous species, both mycorrhizal and nonmycorrhizal (Fig. 4). Calcium ions were released from roots at low pH; the

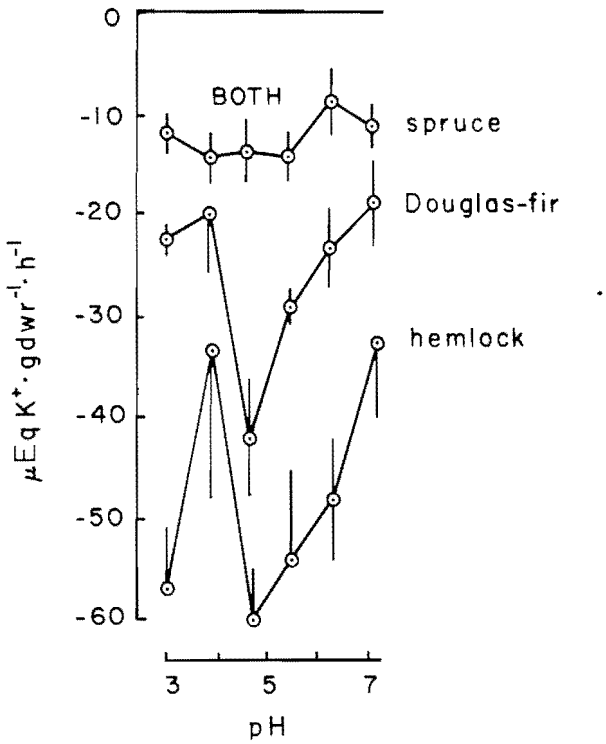

FIG. 5. Flux of K^+ during NH_4^+ uptake as a function of pH. All conventions as in Figs. 1 and 2.

maximum release rate was -27 μEq·gdwr^{-1}·h^{-1}. As pH increased, Ca^{2+} efflux rates decreased until Ca^{2+} uptake occurred above pH 5.5.

Potassium ion

The mycorrhizal association did not affect K^+ efflux, so data were combined (Fig. 5). Efflux of K^+ from roots occurred in all treatments at all pH levels, and was highly variable. Both pH and coniferous species interacted in their effects on K^+ efflux and the interaction was very highly significant. For spruce, efflux rates were low and unaffected by pH. In contrast, Douglas-fir and hemlock K^+ release rates were strongly affected by pH. At pH 4.7, both species had very fast K^+ release with efflux rates decreasing above and below this pH. At pH 3.1, hemlock roots had a very rapid release rate.

Ion fluxes

Because all ions measured were positively charged, uptake rates were added and efflux rates were subtracted in calculating total ion flux. The sums were not zero, suggesting that other ions should be measured. The cumulative ion fluxes for mycorrhizal and nonmycorrhizal seedlings were significantly different only for Douglas-fir. For all three coniferous species, pH effects on total ionic fluxes were very highly significant (Fig. 6). Above pH 4.7, net efflux rates generally decreased with increasing pH. Below pH 4.7, efflux rates increased; this pattern was strongly affected by K^+ fluxes (see Fig. 5). When only nonmycorrhizal seedlings were analyzed, there was no significant differences among coniferous species. When cumulative fluxes of mycorrhizal seedlings were analyzed, significant differences among species were found. Mycorrhizal spruce roots had the least cation efflux (-16 μEq·gdwr^{-1}·h^{-1}) followed by mycorrhizal Douglas-fir (-37 μEq·gdwr^{-1}·h^{-1}) and hemlock (-52 μEq·gdwr^{-1}·h^{-1}) (Table 3).

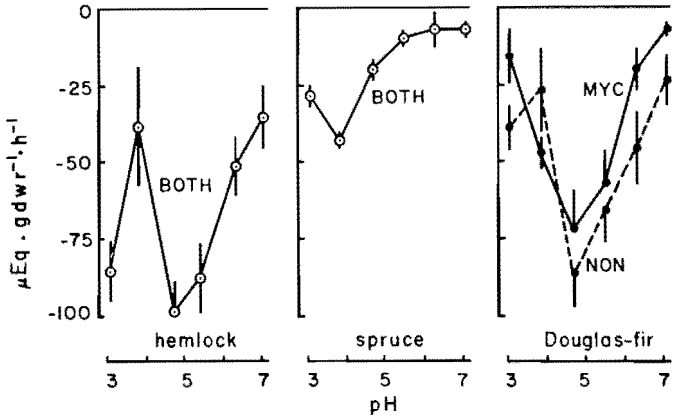

FIG. 6. Measured cation flux (NH_4^+, H^+, Ca^{2+}, and K^+) as a function of pH. All conventions as in Figs. 1 and 2.

Discussion

Solution pH effects on NH_4^+ uptake

The mean NH_4^+ uptake rates measured in this study were 1–9 μEq·gdwr^{-1}·h^{-1} for 9-month-old coniferous seedlings. Similar rates were reported for 2-month-old nonmycorrhizal *Pinus radiata* seedlings, about 7 μEq·gdwr^{-1}·h^{-1} from a 0.1 m*M* NH_4^+ solution, pH not specified (Flewelling 1979). For 2-year-old Douglas-fir seedlings, NH_4^+ uptake rates were about 25 μEq·gdwr^{-1}·h^{-1} (Littke 1982). In contrast, uptake rates for several agricultural species were about 80 μEq·gdwr^{-1}·h^{-1} (Bloom and Chapin 1981). The differences in NH_4^+ uptake rates between agricultural plants and coniferous seedlings may be a result of the greater amount of suberized, less active root mass on coniferous roots. In addition, conifers may have lower uptake rates because they grow slowly and are adapted to forest soils which generally have low levels of available NH_4^+. Chapin (1983) suggested that slow-growing plant species may have lower rates of nutrient uptake, while faster-growing species may have faster rates.

Increasing NH_4^+ uptake with increasing pH is typical of many plant species, including the coniferous seedlings in this study. If competition between H^+ and NH_4^+ for uptake occurs, then NH_4^+ absorption would be least at low pH and would increase as pH increases. For many agricultural plants, maximum cation absorption occurs between pH 5 and 7; below this range, cation absorption is sharply reduced (Moore 1974). Increased H^+ ions in solution may influence the physiochemical environment of the apoplast by protonation resulting in a charge reduction or reversal that could influence NH_4^+ mobility through the apoplast.

Mycorrhizal spruce and Douglas-fir NH_4^+ uptake rates were greatest at pH 6.3 and reduced at 7.1. Although this downward trend in uptake was significant only for Sitka spruce, the trend suggests that there may be a pH optimum around pH 6.3. The reduction in uptake at pH 7.1 for mycorrhizal spruce and mycorrhizal Douglas-fir may have been affected by the fungal symbiont. When *H. crustuliniforme* was grown in buffered liquid medium, optimal growth occurred at pH 5.0 (Littke et al. 1984). If NH_4^+ uptake by the fungus is also maximal at pH 5.0, then this fungus may indeed have decreased the pH optimum for NH_4^+ uptake by mycorrhizal roots. Although Hung and Trappe (1983) reported that the pH optimum for the growth of *H. crustuliniforme* was 7.0, the pH optimum may have been somewhat lower, owing to change in pH of the media with fungal growth.

Mycorrhizal effects on NH_4^+ uptake

Considerable work has been done on mycorrhizal enhancement of nitrogen nutrition, but little of this work pertains to coniferous species (see Bowen and Smith 1981). In an early report on mycorrhizal enhancement of nitrogen uptake, Hatch (1937) found that rates for mycorrhizal *Pinus strobus* were 1.6–2 times greater than the rates for nonmycorrhizal plants. More recently, Littke (1982) reported that mycorrhizal Douglas-fir had 2-fold greater NH_4^+ uptake rates than nonmycorrhizal seedlings. The mycorrhizal seedlings also had lower k_m and higher V_{max} kinetic constants for NH_4^+ uptake. In this study all three coniferous species had higher NH_4^+ uptake rates when mycorrhizal with *Hebeloma crustuliniforme* (rather than when nonmycorrihzal). Because this work was done in well-mixed solutions, the mycorrhizal effects were presumably due to a combination of more surface area for absorption and to more efficient cellular uptake mechanisms.

We recognize that coniferous species are known to form mycorrhizal associations with hundreds of diverse fungal species. These different fungi may have different effects on NH_4^+ uptake than the single fungal species studied here, and these fungi may enhance NH_4^+ uptake rates more or less than *Hebeloma crustuliniforme*. The enhancement of NH_4^+ uptake by *H. crustuliniforme* may be due to the higher NH_4^+ uptake rate of the fungus alone (310 $\mu Eq \cdot gdw^{-1} \cdot h^{-1}$, Littke et al. 1984), as compared with coniferous roots (9 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$, this study).

Ratio of H^+:NH_4^+

The release of H^+ during NH_4^+ uptake reported here is in agreement with many reports of widely observed changes in solution and rhizosphere pH (Smith and Raven 1979; Smiley 1974; Bledsoe and Zasoski 1983; Marschner and Römheld 1983). From our data on coniferous species, the grand mean $H^+:NH_4^+$ ratio was 1.5 and varied with pH from 0.3 at pH 3.1 to 3.0 at pH 7.1. Dejaegere et al. (1981) compiled literature for agricultural species. They found that approximately 1.4 H^+ were released for every NH_4^+ taken up in a pH range of approximately 5–6. The apparently higher $H^+:NH_4^+$ ratio for coniferous species at pH 6 probably resulted from higher cation absorption. Our data suggest that these conifers take up considerably more cations than do agricultural species.

The effects of pH on the $H^+:NH_4^+$ ratio may be related to two factors. First, at higher pH, greater total cation absorption may occur and more H^+ would be released to maintain charge balance. Secondly, at low pH, some H^+ ions would combine with HCO_3^- released by the roots or in solution and would form carbonic acid to varying extents depending upon solution pH (Garrels and Christ 1965). This removal of H^+ by carbonic acid formation would result in an underestimate of H^+ efflux as measured by the pH electrode. The $H^+:NH_4^+$ ratio would correspondingly be lower. Data from additional experiments (Rygiewicz and Bledsoe 1985) suggest that HCO_3^- release by roots is small relative to fluxes of other ions so that this effect would be modest.

Mycorrhizal plants took up NH_4^+ at faster rates than nonmycorrhizal plants, but did not release H^+ ions at faster rates (Figs. 1 and 2). These observations are supported by the $H^+:NH_4^+$ data which show that above pH 4.7 significantly fewer H^+ ions were released by mycorrhizal plants per NH_4^+ taken up (Fig. 3). Mycorrhizae appear to act as rhizosphere buffers. From our solution culture data, we suggest that in soil nonmycorrhizal roots would tend to reduce the rhizosphere pH more than mycorrhizal roots. This ability of mycorrhizae to minimize acidification in the rhizosphere could be important. For example, since both ammonium uptake and phosphate availability are reduced at lower pH, it would be advantageous to minimize rhizosphere acidity.

Charge balance

Ion uptake by plant roots requires maintenance of charge balance (Kirkby 1969). In our study there was net cation release by roots, particularly at low pH for all treatments (Fig. 6). In general, we measured uptake of NH_4^+ and release of H^+, K^+, and Ca^{2+}. Obviously there was movement of other ions so that charge balance could be maintained. To balance the net cation efflux shown by our data, there are three possibilities: (*i*) other cations must be taken up; (*ii*) anions must be released; or (*iii*) a combination of (*i*) and (*ii*).

Sodium, introduced to maintain pH, was the only unmeasured cation. Sodium uptake could help balance cation release, and recent data from our laboratory have shown that Na^+ can be taken up by Douglas-fir roots in significant amounts (Rygiewicz and Bledsoe 1985). If anions were released from the roots, charge balance could also be maintained. Anions present in coniferous roots in sufficient quantities include SO_4^{2-}, $H_2PO_4^-$, HCO_3^-, and organic anions. We measured fluxes of both SO_4^{2-} and $H_2PO_4^-$ for one set of seedlings (hemlock), which had the most rapid rates of cation release (Table 3). At all pH levels, $H_2PO_4^-$ was taken up; mean uptake rate was 0.78 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$, with no differences between mycorrhizal and nonmycorrhizal seedlings. At all pH levels, SO_4^{2-} was released; mean efflux rate was -18 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ for nonmycorrhizal seedlings and -12 $\mu Eq \cdot gdwr^{-1} \cdot h^{-1}$ for mycorrhizal seedlings. Thus $H_2PO_4^-$ had little effect on charge balance, while SO_4^{2-} efflux balanced approximately half the net cation efflux.

We did not measure either HCO_3^- or organic anions in this study; efflux of these ions would also balance cation efflux. In subsequent work we have measured low rates of HCO_3^- efflux by Douglas-fir roots and very little organic anion efflux (Rygiewicz and Bledsoe 1985). Thus it seems likely that efflux of Ca^{2+} and K^+ by the coniferous roots in this study was accompanied both by Na^+ uptake and SO_4^{2-} efflux, while $H_2PO_4^-$, HCO_3^-, and organic anion movements had little effect.

With regard to efflux of K^+ and Ca^{2+} at low pH, we recognize that this must be a short-term phenomenon. Over longer time periods, these roots certainly accumulate both ions. We suggest that this short-term release at low pH is caused by two factors. First, NH_4^+ uptake may be linked with K^+ release. Bledsoe (in Cole 1981) reported that NH_4^+ was rapidly taken up while K^+ was released from Douglas-fir roots in solution culture. Several days later when NH_4^+ was depleted from solution, K^+ was reabsorbed. When solutions were renewed each week, the same pattern was repeated, suggesting a link between NH_4^+ uptake and K^+ release. A second possibility is that some exchange of H^+ for K^+ or Ca^{2+} in the apoplast may have occurred, particularly at a low pH. We believe that it is unlikely that efflux of either K^+ or Ca^{2+} was a result of root damage, acid toxicity, or high salt roots for the following reasons: (*i*) these coniferous roots commonly grow in soils with pH values as low as 3.5; (*ii*) the roots were taking up NH_4^+ even at low pH, an indication that root membranes were functional and undamaged; (*iii*) the roots were not "high salt" roots with respect to either K^+ or Ca^{2+} (Table 2), since Ryan (1983) reported similar K^+ and Ca^{2+} concentrations in values for

solution culture grown roots of Douglas-fir, hemlock, and spruce.

Species comparisons

Nitrogen uptake rates for western hemlock, Douglas-fir, and Sitka spruce are consistent with their relative growth rates (van den Driessche 1971). In the seedling stage western hemlock grows slowly relative to spruce and Douglas-fir. In this study, NH_4^+ uptake rates for hemlock were significantly lower than the rates of Douglas-fir and spruce. The differences in H^+ release and $H^+:NH_4^+$ ratios are also interesting. Western hemlock is known to do well in highly acid, organic substrates and to react poorly to lime and elevated pH (Heilman and Ekuan 1973). Recent studies by Ryan (1983) suggest that hemlock in solution culture can tolerate H^+ but not Al^{3+}, while Douglas-fir has a reverse tolerance. In our studies, hemlock had lower NH_4^+ uptake rates than Douglas-fir, but higher $H^+:NH_4^+$ ratios, suggesting that hemlock may not only tolerate acid conditions but would tend to create acidity in the rhizosphere. Hemlock may have created a niche on acid soils with its slower growth, lower NH_4^+ uptake, and higher H^+ release rates. The higher acidity might reduce competition for available nutrients from bacteria, other fungi, and other plant roots.

Summary

Ammonium uptake by western hemlock, Douglas-fir, and Sitka spruce seedlings was significantly reduced by decreasing pH. This was true of both mycorrhizal and nonmycorrhizal seedlings. This pH effect is particularly significant because, in this study, ammonium was equally available at all pH levels in the well-stirred solutions and ambient pH was not allowed to vary as NH_4^+ uptake occurred. A decreased uptake at decreased pH levels was still observed. While the results from our study cannot sort out the exact nature of the mechanisms involved, the data clearly demonstrate that H^+ directly or indirectly affects NH_4^+ uptake. This H^+ effect has important implications for acid rain studies or studies on development of ecosystems which proceed toward increasing acidity. This research, which has shown that the mycorrhizal fungus *Hebeloma crustuliniforme* enhanced NH_4^+ uptake, supports the concept that mycorrhizae in general benefit plants by increased nutrient uptake. Natural seedlings may be completely mycorrhizal, while the seedlings in this study were 40–50% mycorrhizal. Thus the fungal-mediated enhancement of NH_4^+ uptake may be even greater in the field than in our greenhouse research.

In addition, the three studied coniferous species associate in nature with many other fungal species, which may have different effects on NH_4^+ uptake from the single fungus we studied. In this study, mycorrhizae sometimes lowered the $H^+:NH_4^+$ ratios for coniferous species which suggests that mycorrhizas may act as rhizosphere buffers and achieve enhanced nutrient status for the higher plant–mycobiont system.

Acknowledgements

This project was supported by National Science Foundation grant No. DEB-8004629 to C. S. Bledsoe. We wish to thank C. P. P. Reid and R. L. Edmonds for reviewing this manuscript, and B. B. McInteer of the Los Alamos National Laboratory for the ^{15}N analyses.

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